Growing steers grazing high versus low endophyte (*Neotyphodium coenophialum*)-infected tall fescue have reduced serum enzymes, increased hepatic glucogenic enzymes, and reduced liver and carcass mass[1]

K. R. Brown,* G. A. Anderson,* K. Son,* G. Rentfrow,* L. P. Bush,† J. L. Klotz,‡ J. R. Strickland,‡ J. A. Boling,* and J. C. Matthews*[2]

*Department of Animal and Food Sciences, University of Kentucky, Lexington 40546-0215; †Department of Plant and Soil Sciences, University of Kentucky, Lexington 40546-0312; and ‡Forage-Animal Production Research Unit, USDA-ARS, Lexington, KY 40546-0091

ABSTRACT: It is well established that grazing *Neotyphodium coenophialum*-infected forages results in reduced BW gain and serum prolactin concentrations of cattle. The objective of this study was to determine the potential effects of toxic endophyte-infected tall fescue consumption on blood metabolites, carcass characteristics, and content of proteins critical for AA metabolism in the liver, kidney, and LM tissue of growing steers. Steers grazed a low toxic endophyte (LE; 0.023 μg/g ergot alkaloids) tall fescue-mixed grass pasture (n = 9; BW = 266 ± 10.9 kg; 5.7 ha) or a high toxic endophyte (HE; 0.746 μg/g of ergot alkaloids) tall fescue pasture (n = 10; BW = 267 ± 14.5 kg; 5.7 ha) from June 14 through at least September 11 (≥89 d). No difference was observed for BW ($P < 0.10$) for the overall 85-d growth period. Also, no differences were observed for ribeye area/100 kg of HCW ($P > 0.91$), backfat ($P > 0.95$), or backfat/100 kg of HCW ($P > 0.67$). However, ADG ($P < 0.01$), final BW ($P < 0.05$), HCW ($P < 0.01$), dressing percentage ($P < 0.01$), ribeye area ($P < 0.01$), whole liver wet weight ($P < 0.01$), and whole liver wet weight/100 kg of end BW ($P < 0.01$) were greater for LE steers than HE steers. After 85 d of grazing, serum concentrations of alkaline phosphatase ($P < 0.05$), alanine aminotransferase ($P < 0.01$), aspartate aminotransferase ($P < 0.03$), cholesterol ($P < 0.01$), lactate dehydrogenase ($P < 0.01$), and prolactin ($P < 0.01$) were less for HE than LE steers. At slaughter, hepatic content of cytosolic phosphoenolpyruvate carboxykinase ($P < 0.01$) was greater in HE steers than LE steers. Hepatic content of aspartate aminotransferase ($P < 0.01$) also was greater, whereas renal and LM content were not ($P \geq 0.42$). No differences ($P \geq 0.15$) were observed for hepatic, renal, and LM content of alanine aminotransferase, glutamate dehydrogenase, glutamine synthetase, and 3 glutamate transport proteins. These data indicate that the HE steers displayed classic endophyte toxicity symptoms for growth and blood variables, classic symptoms that were concomitant with novelly identified altered glucogenic capacity of the liver and decreases in carcass characteristics.

Key words: alkaloid, aspartate aminotransferase, bovine, liver, phosphoenolpyruvate carboxykinase

J. Anim. Sci. 2009. 87:748–760
doi:10.2527/jas.2008-1108

INTRODUCTION

Tall fescue (*Festuca arundinacea* Schreb., also called *Lolium arundinaceum*) is a grass commonly used in grazing systems in the United States. *Neotyphodium coenophialum* is an endophytic fungus that infects most tall fescue pastures. The interaction between tall fescue and this endophyte fungus produces alkaloids (Strickland et al., 1993), which aid in drought tolerance and increased forage production (Hill et al., 1991). However, the health and production of cattle grazing tall fescue that contains alkaloids is impaired, resulting in a large negative economic effect on producers (Hoveland, 1993). Clinical symptoms of fescue toxicosis in cattle include but are not limited to decreased BW gain (Porter and Thompson, 1992) and feed intake (Schmidt et al., 1982), reduced reproductive efficiency (Monroe et al., 1988) and milk production (Stuedemann and Hoveland, 1988), increased respiration rates (Browning and

[1]This research was supported by a USDA-ARS Special Cooperative Agreement, the University of Kentucky, and the Kentucky Agricultural Experiment Station (Publ. No. 08-07-058).

[2]Corresponding author: jmatthew@uky.edu

Received April 17, 2008.
Accepted October 10, 2008.

Leite-Browning, 1997) and body temperatures (Hemken et al., 1981), rough hair coats (Bush et al., 1979), and preference for shade.

Common serological signs of fescue toxicosis include decreased alkaline phosphatase (**ALP**; Thompson and Stuedemann, 1993; Davenport et al., 1993), aspartate aminotransferase (**AST**), creatine kinase, lactate dehydrogenase (**LDH**; Dougherty et al., 1991; Cheeke, 1995), alanine aminotransferase (**ALT**; Oliver et al., 2000), and prolactin in rabbits (Daniels et al., 1984), rats (Porter et al., 1985), cattle (Davenport et al., 1993; Schultze et al., 1999), and sheep (Fiorito et al., 1991). However, data from genomic analyses of the effect of fescue toxicosis on gene expression in mouse (Bhusari et al., 2006) and rat (Settivari et al., 2006) liver suggest that changes in serum enzyme concentrations could result from alteration of tissue content of proteins, not just an increased rate of protein release from tissues into the blood. We hypothesized that cattle grazing high toxic endophyte-infected tall fescue would display depressed growth, altered serum analyte profiles, and carcass characteristics (all symptoms of fescue toxicosis), and tissue-specific changes in protein expression of transporters and enzymes critical for AA metabolism and gluconeogenesis, relative to cattle grazing low toxic endophyte-infected tall fescue-mixed grass. Therefore, our experimental objective was to compare these variables between steers exhibiting fescue toxicosis vs. control steers.

MATERIALS AND METHODS

All experimental procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Animals

Nineteen predominately Angus beef steers were denied access to feed and water for 14 h, weighed, and subdivided into 2 groups based on BW and randomly allotted (d 0) to 1 of 2 pastures that lacked shade. Pastures were within 500 m of each other and are part of the University of Kentucky Agricultural Research Center, located in Woodford County, KY. Steers were assigned to graze low toxic endophyte tall fescue-mixed grass (**LE**, n = 9, 5.7 ha) or a high toxic endophyte infected tall fescue (**HE**, n = 10, 5.7 ha). All steers had ad libitum access to fresh water and mineral supplement (Ca, minimum 13.0, maximum 15.0%; P, 6.2%; NaCl, minimum 17.0, maximum 19.5%; Mg, 3.0%; S, 1.0%; K, 0.8%; Zn, 2,300 μg/g; Mn, 2,200 μg/g ; Cu, 1,450 μg/g; I, 45 μg/g; Co, 15 μg/g; Se, 29 μg/g; vitamin A, 661 IU/g; vitamin E, 0.276 IU/g; as-fed). Steers grazed for 85 d. On d 86, steers were denied access to water and forage for 12 h, after which BW were taken to determine ADG. Steers were returned to their respective pastures and randomly allotted to slaughter date based on forage treatment. Slaughter was conducted on d 89, 91, 98, 103, and 105.

Pasture Sampling and Analysis

On d 37, 59, 88, and 109 of the study, leaf blades suitable for grazing were detached from each pasture for ergot alkaloid (ergovaline, ergovalinine, lysergic acid, and isolysergic acid) determination, proximate analysis, and mineral content. Briefly, samples were obtained systematically from approximately 30 sites in each pasture using a knife to cut the forage at approximately 2 cm above soil level. Samples were immediately placed into individual plastic bags and then stored on ice during transportation to our laboratory. All samples were frozen and stored at −20°C. Analysis of ergot alkaloids was performed as described previously (Yates and Powell, 1988), and isolysergic acid was quantified with a lysergic acid standard. Proximate analysis and mineral content were determined by a commercial laboratory (Dairy One Forage Lab, Ithaca, NY).

Determination of Temperature Humidity Index, and Heat Stress

Weather data, temperature (temp), and relative humidity (rh), were recorded by an automated weather station of the University of Kentucky Agricultural Weather Center. This station is located at the University of Kentucky Agricultural Research Center and is approximately equidistant (150 m) from each pasture. Weather data were measured every hour and used to calculate the temperature-humidity index (**THI**; adapted from Thom, 1959; Hahn, 1999; Amundson et al., 2006):

$$\text{THI} = (0.8\ \text{temp}) + [(\text{rh}/100)\ (\text{temp} - 14.4)] + 46.4.$$

Temperature-humidity index values serve as the basis for the Livestock Safety Index (LCI, 1970), which are classified as follows: normal, ≤74; alert, 75 to 78; danger, 79 to 83; emergency ≥84. A THI greater than 74 corresponds to a period when cattle are above their thermoneutral zone (Mader et al., 2002) and heat recovery threshold (Hahn, 1999; Hahn and Mader, 1997). Therefore, steers were considered to be heat stressed on a given day when THI values were greater than 74.

Blood Collection

Jugular venous blood samples were collected by venipuncture on d 85. For preparation of plasma, 16 mL of blood was collected in EDTA-containing (0.9375 mg/mL) blood collection tubes (Becton Dickinson, Franklin Lakes, NJ). For serum, 16 mL of blood was collected in serum blood collection tubes without an anticoagulant. For whole blood, 2 mL of blood was collected in EDTA-containing (2.7 mg/mL) blood collection tubes, Becton Dickinson). Plasma and sera were recovered by refrigerated centrifugation at 3,000 × *g* for 10 min at 4°C and stored at −80°C. Plasma samples were analyzed for ammonia-N by modifications of the L-Glu dehydro-

genase assay (Da Fonesca-Wollheim, 1973) using the Konelab 20XTi analyzer (Thermo Electron Corp., Vantaa, Finland). The sensitivity of this assay is 0.010 m*M*, and an interassay CV of 11.0% is typically realized. For this experiment, plasma ammonia concentrations were determined in a single assay event. The intraassay CV was 7.4%. Serum prolactin analysis (Bernard et al., 1993) was conducted (F. N. Schrick Laboratory, Johnson Animal Research and Teaching Unit, University of Tennessee, Knoxville). All other serum analytes, minerals, and blood cell types were analyzed by the University of Kentucky Livestock Disease Diagnostic Center (**UKLDDC**). For serum enzymes, the following specific activities were assayed: ALP, E.C. 3.1.3.1; ALT, E.C. 2.6.1.2; AST, E.C. 2.6.1.1; γ-glutamyltransferase, E.C. 2.3.22; creatine kinase, E.C. 2.7.3.2; LDH, E.C. 1.1.1.27.

Slaughter, Tissue Collection, and Carcass Evaluation

As noted above, steers were slaughtered over a 17-d period, on d 89 (n = 1, LE; n = 2, HE), 91 (n = 2, LE; n = 2, HE), 98 (n = 2, LE; n = 2, HE), 103 (n = 2, LE; n = 2, HE), and 105 (n = 2, LE; n = 2, HE) of the study. Steers were transported from the University of Kentucky Animal Research Center to the University of Kentucky Meat Laboratory 1 to 3 h before slaughter, where they had ad libitum access to water until time of slaughter. Body weight was determined and steers were stunned by captive-bolt gun followed by exsanguination. A mid-lateral incision was made to gain access to the abdominal and thoracic cavity for tissue collection. Serially, the liver, heart, and the right kidney were removed. The gall bladder was removed and liver weight recorded. Liver tissue was collected from the mid-lower right lobe. Renal fat was dissected from around the kidney, the kidney weighed, and a cross-section (encompassing cortex and medulla tissue) sample taken from a middle lobe. After the HCW was recorded, tissue from the LM was collected from between the 12th and 13th rib from the left side of the carcass. All tissue samples were placed in foil packs, snap-frozen in liquid nitrogen, and stored at −80°C. After 24 h postmortem, carcass evaluations (ribeye area and fat depth at 12th rib) were conducted on the right side of the carcass according to USDA standards (USDA, 1997).

Immunoblot Analysis

Approximately 1 g of liver, kidney, and LM were homogenized on ice for 30 s (setting 11, Polytron Model PT10/35, Kinematic Inc., Lucerne, Switzerland) in 7.5 mL of 4°C sample extraction buffer solution [0.25 m*M* sucrose, 10 m*M* HEPES-KOH pH 7.5, 1 m*M* EDTA, and 50 μL of protease inhibitor (Sigma, St. Louis, MO)]. Protein was quantified by a modified Lowry assay, using BSA as a standard (Kilberg, 1989). Proteins were separated using 12% SDS-PAGE and electrotransferred to a 0.45-μm nitrocellulose membrane (BioRad, Hercules, CA) as described previously (Howell et al., 2001, 2003), except that proteins were separated using a 12% acrylamide gel instead of 7.5%. Blots were stained with fast-green and the relative amount of stained protein per lane/sample determined by densitometric analysis (see below).

The relative tissue content of specific proteins in liver, kidney, and LM was evaluated using a standard immunoblot protocol as described previously (Howell et al., 2001, 2003). Relative contents of ALT, AST, glutamate dehydrogenase (**GDH**), glutamate transporter-1 (**GLT-1**), excitatory AA carrier 1 (**EAAC1**), and glutamate transporter-associated protein 3–18 (**GTRAP3–18**) were evaluated in all 3 tissues. The relative content of glutamate synthetase (**GS**) was determined in liver and kidney, whereas GS and cystolic phosphoenolpyruvate carboxykinase (**PEPCK-C**) were evaluated only in liver. For the detection of GLT-1, EAAC1, GTRAP3–18, GDH, and PEPCK-C, blots were hybridized with 5 to 10 μg of IgG anti-rat GLT-1 polyclonal antibody (Affinity BioReagents, Golden, CO), 1 μg of IgG anti-human EAAC1 polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA), 4 μg of IgG anti-human GTRAP3–18 (Abcam Inc., Cambridge, MA), 85 μg of IgG anti-bovine GDH (United States Biological, Swampscott, MA), and 0.59 μg of IgG anti-human PEPCK-C (Abcam Inc.), respectively, per mL of blocking solution [1% nonfat dry milk (wt/vol; Carnation, Nestle, Solon, OH) in 30 m*M* Tris-Cl (pH 7.5), 200 m*M* NaCl, 0.1% Tween 20 (vol/vol)] for 1.5 h at room temperature with gentle rocking. For AST and ALT detection, blots were hybridized with 20 μg of IgG anti-mouse AST (Fitzgerald Industries International Inc., Concord, MA) and 40 μg of IgG anti-porcine ALT (United States Biological), respectively, per milliliter of blocking solution [1.5% nonfat dry milk (wt/vol) in 30 m*M* Tris-Cl (pH 7.5), 200 m*M* NaCl, 0.1% Tween-20] for 1.5 h at room temperature with gentle rocking. Lastly, GS was probed using 1.25 μg of IgG anti-sheep polyclonal antibody (BD Biosciences, San Jose, CA) per milliliter of blocking solution [5% nonfat dry milk (wt/vol), 10 m*M* Tris-Cl (pH 7.5), 100 m*M* NaCl, 0.1% Tween 20 (vol/vol)] for 1 h at 37°C with gentle rocking.

All protein-primary antibody binding reactions were visualized with a chemiluminescence kit (Pierce, Rockford, IL) after hybridization of primary antibodies with horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham, Arlington Heights, IL; GLT-1 and EAAC1, 1:5,000; GDH, 1:7,500; and PEPCK-C, 1:8,000); horseradish peroxidase-conjugated goat anti-mouse IgG (BD Biosciences; GS, 1:5,000); horseradish peroxidase-conjugated rabbit anti-sheep IgG (Santa Cruz Biotechnology; ALT and AST, 1:5,000); or horseradish peroxidase-conjugated donkey anti-goat IgG (Santa Cruz Biotechnology; GTRAP3–18, 1:5,000).

Densitometric analysis of immunoreactive products was performed as described previously (Howell et al.,

2003; Fan et al., 2004). Briefly, after exposure of autoradiographic film (Amersham), digital images of all observed immunoreactive species were recorded and quantified (Yamin et al., 1996; Dehnes et al., 1998; Ding et al., 1998) using the BioRad Versadoc imaging system and the Quantity One Program (version 4.2.3, BioRad). A single immunoreaction product was assessed for treatment effect by densitometric analysis as follows: AST, 41kDa; PEPCK, 72 kDa; ALT, 23 kDa; GDH, 55 kDa; GS, 43 kDa; GLT-1, 74 kDa; EAAC1, 69 kDa; and GTRAP3–18, 41 kDa. The linearity of antibody-ligand immunoreactions and densitometry were validated using immunoblots containing protein gradients (data not shown). Data were collected as arbitrary densitometric units and then were corrected for unequal loading, transfer of proteins, or both by normalization to densitometric values of Fast-Green-stained (Fisher Scientific, Pittsburgh, PA) proteins common to all immunoblot lanes/samples. For all results, densitometric values were normalized to LE steers by obtaining an average control densitometric value and dividing all results by this value. Digital images were prepared with PowerPoint (Microsoft PowerPoint 2003, Bellevue, MA).

Statistical Methods

Data are presented as least square means (±SEM). All measured experimental variables between steers displaying fescue endophyte toxicosis (HE) and control steers (LE) were evaluated by ANOVA, using the MIXED procedure (SAS Inst. Inc., Cary, NC). Steers were the individual experimental units. The statistical model used fescue toxicosis as the fixed effect. Class variables were fescue toxicosis and steer, with steer included in the random statement. Kenward-Roger adjustment was used to calculate the denominator df (Kenward and Roger, 1997).

RESULTS AND DISCUSSION

Experimental Model: Induction of Fescue Toxicosis

The goal of this comprehensive project was to induce fescue toxicity via ergot alkaloid exposure in growing Angus steers using a typical commercial regimen (HE; summer-long grazing of high toxic endophyte infected tall fescue) and to then determine if altered variables associated with fescue toxicosis (altered serum analyte profiles and depressed growth) were accompanied by tissue-specific changes in protein expression of transporters and enzymes critical for AA metabolism and glucogenesis, relative to cattle grazing low toxic endophyte infected tall fescue-mixed grass.

Perhaps the most consistent clinical marker of fescue toxicosis in cattle is a depressed concentration of serum prolactin (Goetsch et al., 1987; Davenport et al., 1993; Strickland et al., 1993). Typically, a decrease in serum prolactin results from inhibition of prolactin secretion due to stimulation of D2-type dopamine receptor-mediated responses by dopamine (Peters et al., 1981; Ben-Jonathan et al., 1989; Freeman et al., 2000). However, in cattle with fescue toxicosis, it has been suggested

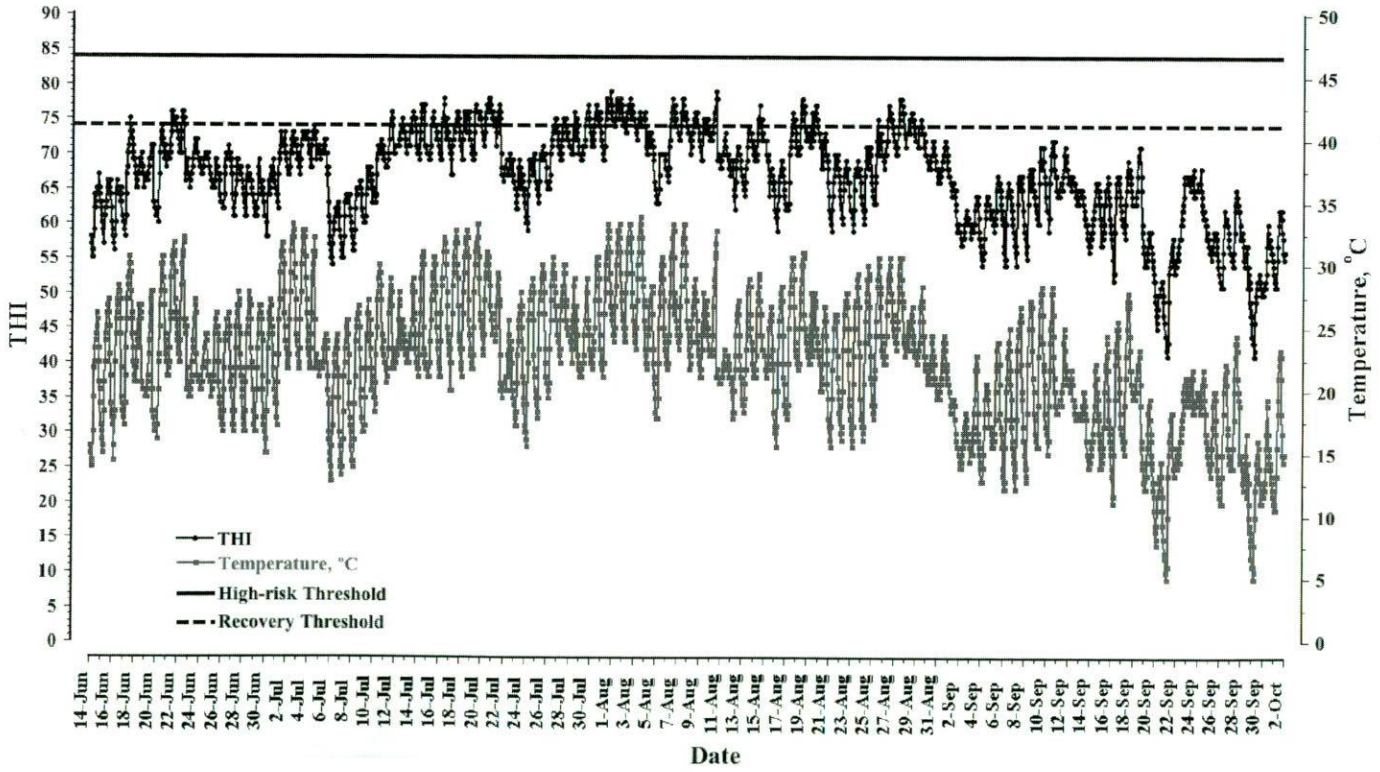

Figure 1. Hourly temperature-humidity index (THI) values and temperatures for the 88-d common grazing (June 14 to September 10) and 17-d slaughter (September 11 to September 27) periods. The high-risk (THI ≥ 84) and recovery (THI ≤ 74) thresholds were designated as per LCI (1970) and Hahn and Mader (1997).

(Strickland et al., 1992) that it is the interaction between D2-type receptors and specific alkaloids in tall fescue that cause suppression of prolactin secretion. In the current study, serum prolactin concentrations (3.6 ng/mL) in HE steers were 10.0% ($P < 0.01$) that of the LE steers. The magnitude of prolactin suppression observed for HE steers is common (Schillo et al., 1988; Nihsen et al., 2004), and seemingly reflects the greater ergot alkaloid load of the forage being grazed and a greater level of ergot alkaloid-mediated toxicity in the animal.

A common symptom of cattle with fescue toxicosis is the presence of a long and rough coat of hair (Hemken et al., 1984). Consistently, steers grazing the HE forage had long rough hair, which should have been shed by this time of year, whereas those grazing the LE forage for 85 d had smooth, relatively short hair coats. Uniquely evident for the HE steers was caked mud on the coats (photos not shown). Consumption of toxic endophyte infected fescue often causes an increase in body temperature (Hemken et al., 1981). Although body temperature was not measured, the caked mud on hair coats of the HE steers is evidence of steers wallowing in an attempt to reduce body temperature and is consistent with previous studies in cattle exhibiting fescue toxicosis (Hemken et al., 1984; Beck et al., 2008).

For the common grazing period, d 0 to 88 (June 14 to September 10), steers grazing both pastures experienced an LCI (1970) classification of alert (THI of 75 to 78) for at least 1 h during 37 d (Figure 1). In addition, on 1 of those days (August 10), the steers experienced a classification of danger (THI of 79). During blood collection on d 85 (September 7) and the 17-d slaughter period (September 11 to September 27), the LCI (1970) classification was normal (THI $\leq$ 74).

Analysis of ergot alkaloid concentrations between the 2 forages revealed that the HE steers were exposed to 25 and 21 times more ergovaline/ergovalinine and lysergic acid/isolysergic acid, respectively, than were the LE steers (Table 1). However, forage composition (DM, CP, ADF, and crude fat %) did not differ. The HE forage contained less ($P \leq 0.03$) lignin (25%) and NDF (3.3%) and tended to contain more TDN (2.67%, $P = 0.10$). In terms of mineral content, HE contained more ($P \leq 0.01$) ash (6.4%) and K (22%), and less ($P \leq 0.02$) Ca (39%), Mg (32%), and S (8%) than the LE forage.

The ADG (0.40 kg) of steers grazing HE was 31% less ($P < 0.01$) over the first 85 d of the experimental period than for steers grazing LE forage (0.58 kg). Similar reductions in ADG for steers grazing endophyte-infected tall fescue pastures are often observed, ranging from 24% (Stuedemann et al., 1986) to 66% (Crawford et al., 1989). In addition, accounting for the weight of the mud on the hair coat of HE steers would exacerbate the difference in final BW and ADG in favor of HE over LE steers. As indicated by reduced plasma prolactin, longer hair and mud accumulation on hair coats, and decreased ADG, steers grazing the HE forage for 85 d exhibited symptoms of the classic summer slump phenomenon associated with tall fescue toxicosis.

Table 1. Proximate, mineral, and alkaloid analysis of composited low endophyte (LE) and high endophyte (HE) pasture samples (DM basis)[1]

	Pasture treatment			
Item	LE	HE	SEM	*P*-value
Proximate analysis, %				
DM	24.1	24.6	1.1	0.802
CP	17.34	17.64	0.49	0.671
TDN	58.33	59.83	0.62	0.099
ADF	32.09	32.78	0.44	0.362
NDF	60.59	58.63	0.58	0.026
Crude fat	3.42	3.43	0.12	0.961
Lignin	6.25	5.01	0.27	0.003
Mineral analysis, %				
Ash	8.3	8.8	0.1	0.012
Ca	0.75	0.46	0.03	<0.0001
P	0.33	0.32	0.01	0.738
Mg	0.25	0.17	0.01	<0.0001
K	2.09	2.55	0.07	<0.0001
S	0.25	0.23	0.01	0.017
Alkaloid analysis, μg/g				
Ergovaline	0.0133	0.3225	0.0285	<0.0001
Ergovalinine	0.0033	0.1992	0.0254	<0.0001
Lysergic acid	0.0017	0.0650	0.0078	<0.0001
Isolysergic acid	0.0050	0.1592	0.0135	<0.0001

[1]Proximate, mineral, and alkaloid analysis values are an average of 4 samples collected over a 72-d period and presented on a DM basis. Samples were obtained systematically from approximately 30 sites in each pasture, using a knife to cut the forage at approximately 2 cm above soil level. Data are presented as least squares means (±SEM).

Table 2. Serum and plasma analytes of steers grazing low endophyte (LE)- or high endophyte (HE)-infected forages[1]

	Treatment				
Item[2]	LE	HE	SEM[3]	*P*-value	Reference range
Prolactin, ng/mL	36.0	3.6	2.6	<0.0001	—
ALP, U/L	159.8	109.1	17.0	0.045	100.0–500.0[4]
ALT, U/L	32.6	27.3	1.0	0.001	11–40[5]
AST, U/L	71.2	60.6	3.3	0.030	0–160[4]
AST/ALT ratio	2.20	2.23	0.12	0.902	—
Ammonia,[6] m*M*	0.045	0.062	0.006	0.060	—
Blood urea nitrogen, mg/100 mL	16.2	16.4	0.8	0.865	5.0–27.0[4]
Albumin, g/100 mL	3.33	3.14	0.07	0.051	2.30–3.70[4]
Globulin, g/100 mL	3.83	3.86	0.11	0.863	3.0–3.5[4]
Albumin/globulin	0.90	0.83	0.04	0.179	0.80–1.00[4]
γ-Glutamyltransferase, U/L	10.6	9.2	0.7	0.158	2.0–20.0[4]
Total bilirubin, mg/100 mL	0.2	0.2	0.1	1.000	0.0–0.5[4]
Total protein, g/100 mL	7.17	7.00	0.10	0.259	6.50–7.50[4]
Creatinine, mg/100 mL	1.23	1.23	0.06	0.965	1.00–2.00[4]
Blood urea nitrogen:creatinine	13.23	13.45	0.58	0.788	—
Creatine kinase, U/L	223.4	144.8	23.8	0.028	100.0–650.0[4]
Glucose, g/100 mL	78	70	3	0.094	40–100[4]
LDH, U/L	1,063	821	50	0.003	692–1,445[5]
Triglycerides, g/100 mL	30	31	3	0.735	—
Cholesterol, mg/100 mL	107	76	4	<0.0001	62–193[4]

[1]Data are presented as least squares means (±SEM) of LE (n = 9) and HE (n = 10) treatments.
[2]Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.
[3]Most conservative error of the mean.
[4]Taken from the University of Kentucky Livestock Disease Diagnostic Laboratory (Lexington, KY).
[5]Taken from Kaneko et al. (1997).
[6]Plasma.

Biochemical and Clinical Blood Profiles Indicate a Suppressed Metabolic Capacity for HE Steers

To gain insight into potentially altered metabolic capacities that could account for the reduced ADG of HE cattle, profiles of serum enzymes and other blood constituents were compared between HE and LE steers (Table 2). The observation that serum albumin concentrations were not elevated (were within reference values; UKLDDC) for either group (HE vs. LE), indicates that steers were not dehydrated. Similarly, the observation that the absolute values of all measured serum enzymes and analytes were within normal clinical reference values (Table 2; Kaneko et al., 1997; UKLDDC) indicates that steers from both groups did not have detectable tissue necrosis. However, relative to steers grazing LE forages, steers grazing HE forages had decreased ($P \leq 0.05$) concentrations of serum ALP (68%), ALT (16%), AST (15%), LDH (33%), cholesterol (29%), albumin (6.6%), and creatine kinase (35%; Table 2). In contrast, steers grazing HE forage had a 38% increase in ($P = 0.06$) serum ammonia concentrations compared with LE.

Although others have recorded similar patterns of reduced serum enzymes in steers with fescue toxicosis (Thompson and Stuedemann, 1993; Oliver, 1997; Schultze et al., 1999; Nihsen et al., 2004), little discussion has been given to the physiological causes and consequences of these metabolic profiles. However, Schultze et al. (1999) suggested that the decreased growth of cattle with fescue toxicosis is linked moderately to decreased activity of intestinal and bone ALP isoenzymes because of actual loss of mucosal cells in the intestine and decreased osteoblast activity. Others have observed similar decreases in serum ALT, AST, and LDH concentrations (Dougherty et al., 1991; Cheeke, 1995; Nihsen et al., 2004) in cattle consuming endophyte-infected forages as seen in the current study. Although not discussed in such terms, these decreases presumably reflect either a decreased rate of enzyme shedding, decreased enzyme content (expression), or both, by liver, kidney, heart, and skeletal muscle tissues of HE steers. For example, the primary source of serum ALT is thought to be from the cytoplasm of hepatocytes (Fleisher and Wakim, 1963a; Tennant, 1997; Turk and Casteel, 1997), whereas serum AST represents mitochondrial turnover by hepatocytes (Cardinet et al., 1967; Harris, 1997; Tennant, 1997) and striated muscle (Fleisher and Wakim, 1963b; Wakim and Fleisher, 1963; Tennant, 1997). Thus, an increase in the AST:ALT represents a greater alteration of striated muscle function than of hepatic function. In the present study, the concentrations of serum AST and ALT in HE steers were decreased ($P \leq 0.03$) relative to LE steers in similar proportions (15 and 16%, respectively). Consequently, the AST:ALT ratio did not change or differ. Thus, the decrease in the serum of both AST and ALT in HE steers likely reflects

a decreased relative concentration of these enzymes by these tissues, or reduced tissue mass, but likely not an elevated rate of tissue turnover.

The 38% increase in serum ammonia concentration of HE steers suggests that HE steers had a reduced glutamine synthetase-mediated ammonia capture capacity, reduced urea synthesis capacity, or some combination of these metabolic factors (Häussinger, 1986; Lobley and Milano, 1997). Because blood urea N concentrations did not differ, hepatic urea synthetic capacities likely did not differ between HE and LE steers. Instead, HE steers may have had an impaired hepatic glutamine/glutamate cycle or a reduced ammonia recycling capacity by other tissues.

The 6% decrease ($P = 0.05$) in serum albumin of HE-treatment steers, but not in globulin or in the albumin:globulin, indicates that hepatic albumin synthetic capacity of HE was reduced relative to LE steers and not representative of liver necrosis (Stockham and Scott, 2002). Similarly, the findings that serum γ-glutamyltransferase, bilirubin, and total protein values were not affected by grazing treatment supports the concept that HE steers did not suffer from general hepatobiliary insults (Tennant, 1997).

Neither serum creatinine (principally a product of muscle metabolism) nor blood urea nitrogen:creatinine concentrations were affected by grazing treatment (Table 2). These findings, plus the normal serum albumin values, indicate that steers from both groups had normal renal glomerular filtration function (Finco, 1997). However, HE steers had a 35% decrease ($P = 0.03$) in serum creatine kinase. Elevated serum creatine kinase is an indicator of striated muscle (heart and skeletal) trauma. However, creatine kinase concentrations were decreased, not elevated, in HE steers. These findings indicate that content of creatine kinase in skeletal muscle (and perhaps cardiac) was suppressed (possibly due to decreased cellular energy levels), or that the size of these tissues was smaller, in HE vs. LE steers.

Because all steers experienced the same heat stress load, these findings of indirect measurements for metabolic status of tissues suggest that cattle grazing the HE forage were in a state of reduced protein and metabolic capacity, or similarly, a state of increased AA oxidation, compared with those grazing forage with decreased alkaloid concentrations. The reduced albumin (Chan et al., 2003) and increased serum ammonia concentrations in the serum of HE steers are indicative of homeostatic control to preserve carbon skeletons for gluconeogenesis (Groff and Gropper, 2000) as evidenced by the tendency ($P = 0.09$) for decreased serum glucose concentrations in HE steers.

Lactate dehydrogenase isozymes (especially LDH1) are ubiquitously expressed (and presumably shed into blood) by mammalian tissues (Brancaccio et al., 2007), especially by glycolytic skeletal muscles (Duehlmeier et al., 2007; Huber et al., 2007). Therefore, the coincidental 15.4% reduction in ribeye area and 33% decrease ($P < 0.01$) in serum LDH concentrations of HE steers appears to be consistent with a decreased capacity for flow of muscle pyruvate carbons into lactate and a potentially compromised hepatic capacity to convert muscle lactate into pyruvate for gluconeogenesis.

Energy metabolism in the form of altered capacity for lipid metabolism also may have been altered (Cincotta and Meier, 1989; Barnett et al., 1991) in HE steers. Although serum triacylglyceride concentration was not different, serum cholesterol was reduced ($P < 0.01$) 29% in HE vs. LE cattle (Table 2). A decrease in serum cholesterol is interpreted as a stress response due to a change in lipid metabolism (Realini et al., 2005) or fat necrosis in cattle with long-term exposure to endophyte-infected tall fescue (Stuedemann and Hoveland, 1988). A relationship between endophyte toxicity and necrotic adipose tissue has been reported (Stuedemann and Hoveland, 1988). Increased body temperature, rough hair coats, and decreased growth performance are concomitant measures of fat necrosis in cattle grazing toxic high endophyte-infected fescue (Stuedemann et al., 1975; Hoveland et al., 1983; Stuedemann and Hoveland, 1988). Although we did not measure body temperature of steers in the present trial, the HE-exposed steers had long, rough hair coats and decreased ADG relative to steers grazing the LE forage. However, there was no indication of necrosis in gastrointestinal tract adipose tissue of HE or LE steers.

As discussed above, the serum prolactin concentrations of the HE steers were only 10.0% of the LE steers. Not only do prolactin receptors regulate serum prolactin concentration, the receptors are also involved in the regulation of solute and water transport (Shennan, 1994). Therefore, serum minerals were measured and compared between steers grazing the HE and LE forages. Although Cl, P, and Na concentrations did not differ ($P \geq 0.27$, Table 3), serum Ca was decreased ($P = 0.02$) 4.2%, whereas K concentration was increased 12% ($P = 0.03$) in HE steers. Because prolactin is thought to stimulate Ca transport across intestinal epithelia (Pahua and DeLuca, 1981), the finding that serum Ca was reduced in HE steers (which had decreased serum prolactin concentrations) may reflect in part an impaired Ca uptake capacity by intestinal epithelia due to decreased prolactin concentrations. Simultaneously, the reduced serum Ca of HE steers may also reflect the decreased (39%) content of Ca found in the HE vs. LE forage (Table 1). The finding that HE steers had elevated serum K is inconsistent with the understanding that prolactin tends to stimulate K resorption by kidney proximal tubules (Stier et al., 1984). However, the elevated concentration of serum K in HE steers is consistent with the greater (22%) concentrations of K in the HE vs. LE forages. From these findings, it is clear that much remains to be discerned about alkaloid consumption, serum prolactin, and the regulation of serum mineral concentrations in cattle grazing tall fescue.

Because prolactin also is known to affect humoral and cellular immune responses (Freeman et al., 2000), the effect of grazing HE vs. LE forages on presence of blood

Table 3. Serum minerals of steers grazing low endophyte (LE)- or high endophyte (HE)-infected forages[1]

	Treatment				
Item	LE	HE	SEM[2]	*P*-value	Reference range
Ca, mg/dL	10.3	9.8	0.12	0.024	9–12[3]
Cl, mmol/L	106.0	106.5	0.45	0.428	97–111[3]
P, mg/dL	6.6	6.1	0.29	0.275	4–7[3]
K, mmol/L	4.4	4.9	0.16	0.030	3.9–5.8[3]
Na, mmol/L	135.9	135.1	0.76	0.460	132–152[3]

[1]Data are presented as least squares means (±SEM) of LE (n = 9) and HE (n = 10) treatments.
[2]Most conservative error of the mean.
[3]Taken from the University of Kentucky Livestock Disease Diagnostic Laboratory (Lexington, KY).

cell types was evaluated (Table 4). Although forage treatment did not affect relative amounts of total blood cells (packed cell volume) or immune system-related cell types, steers grazing the HE forage had 7.1% more ($P = 0.06$) red blood cells (**RBC**). Given that prolactin is thought to stimulate RBC production (Socolovsky et al., 1998), but that prolactin concentrations in HE steers were only about 10% of LE steers, this result was somewhat surprising. However, Oliver et al. (2000) also observed an increase in RBC concentration of similar magnitude as found in our steers, a finding that also was concomitant with decreased serum prolactin.

HE Steers Have Relatively Smaller Livers, but Not Kidneys or Hearts

As noted above, HE steers displayed differences in the amount of specific enzymes present in serum. Specifically, the principal sources of these enzymes are (Sattler and Fürll, 2004): ALT, cytosol of hepatocytes; AST, mitochondria of hepatocytes and skeletal and cardiac muscle; creatine kinase, skeletal and cardiac muscle. Because the relative amounts of ALT, AST, and creatine kinase in serum are thought to be constantly shed from these tissues, the wet and final BW-adjusted weights of heart and liver were measured at slaughter (d 89 through d 105) and compared between HE vs. LE steers (Table 5). In addition, because of its importance to AA metabolism, the potential effect of HE vs. LE treatment on kidney mass also was determined. For heart and kidney, tissue weight, and tissue weight/100 kg of final BW did not differ between HE and LE treatment groups. In contrast, liver weight ($P < 0.01$) and whole liver weight/100 kg of final BW ($P < 0.01$) were 10 and 6.6% less in the HE than LE steers, respectively. The finding that steers exposed to increased concentrations of endophyte have reduced liver weights, but not kidney masses, also has been observed in rats fed diets containing endophyte-infected tall fescue seed (Chestnut et al., 1992; Settivari et al., 2006).

Effect of Grazing HE Forage on Relative Content of AA Enzymes and Transporters

The decreased size of the livers of HE steers suggests that if rate of enzyme release from the liver was constant, then possessing a smaller liver would result in a proportionate decrease in serum concentrations. However, the liver weight for HE steers was only 10 (whole weight) to 6.6% (adjusted to BW) less than for LE steers, whereas the serum concentrations of ALT, AST, and LDH were 16, 15, and 33% less (respectively) than

Table 4. Blood cell types of steers grazing low endophyte (LE)- or high endophyte (HE)-infected forages[1]

	Treatment				
Item	LE	HE	SEM[2]	*P*-value	Reference range
Red blood cells, $1 \times 10^6/\mu L$	8.49	9.09	0.22	0.061	5.0–10.0[3]
Hemoglobin, g/dL	11.1	11.1	0.3	0.952	8.0–15.0[3]
Packed cell volume, %	32.3	32.3	1.0	0.975	24.0–46.0[3]
White blood cells, $1 \times 10^3/\mu L$	9.22	9.35	0.72	0.902	4.0–12.0[3]
Neutrophils, $1 \times 10^3/\mu L$	3.65	3.80	0.39	0.784	0.06–4.00[4]
Lymphocytes, $1 \times 10^3/\mu L$	5.27	5.19	0.47	0.896	2.5–7.5[4]
Monocytes, $1 \times 10^3/\mu L$	0.36	0.21	0.07	0.119	0.0–0.9[4]
Eosinophils, $1 \times 10^3/\mu L$	0.11	0.21	0.09	0.404	0.0–2.4[4]

[1]Data are presented as least squares means (±SEM) of LE (n = 9) and HE (n = 10) treatments.
[2]Most conservative error of the mean.
[3]Taken from the University of Kentucky Livestock Disease Diagnostic Laboratory (Lexington, KY).
[4]Taken from Duncan et al. (1994).

Table 5. Whole and BW-adjusted heart, kidney, and liver weights of steers grazing forages containing low endophyte (LE)- and high endophyte (HE)-infected forages[1]

	Treatment			
Item	LE	HE	SEM[2]	*P*-value
Weight (wet)				
Heart, g	1,395.6	1,368.6	55.3	0.728
Heart,[3] g/100 kg of BW	413.0	437.6	13.2	0.194
Kidney, g	411.1	373.0	18.4	0.152
Kidney,[3] g/100 kg of BW	1.22	1.19	0.04	0.572
Liver, g	4,107.1	3,563.2	107.01	0.002
Liver,[3] g/100 kg of BW	1,214.9	1,138.9	16.0	0.003

[1]Cattle grazed pasture on for 89 to 105 d before slaughter. Data are presented as least squares means (±SEM) of LE (n = 9) and HE (n = 10). The mean end BW differed ($P = 0.05$) at slaughter and was 313 ± 9.0 kg for HE and 338 ± 8.1 kg for LE.

[2]Most conservative error of the mean.

[3]Based on BW at time of slaughter.

for LE steers, whereas GGT did not differ. Therefore, it seems reasonable to suggest that differences in liver size alone solely did not account for observed differences in serum enzyme concentrations.

To determine if HE-associated differences in concentrations of enzymes measured in serum also differed in tissues thought to contribute to serum concentrations, the relative content of ALT and AST in liver and kidney tissue was evaluated by immunoblot analysis (Table 6). In the liver, no treatment effect on ALT content was found. In contrast, AST content was increased ($P < 0.008$) 56% in steers grazing HE vs. those grazing LE forages (Table 6). The increased content of AST in hepatic tissue of HE steers reflects an increased potential to either shunt aspartate carbons into the tricarboxylic acid (**TCA**) cycle to shunt TCA (oxaloacetic acid; **OAA**) carbons into aspartate.

To determine if an increased AST content indicates an increase in OAA or aspartate production, the relative content of PEPCK-C protein was determined. Cytosolic phosphoenolpyruvate carboxykinase content was increased ($P < 0.01$) 90% in the liver of HE vs. LE grazing steers (Table 6). This finding is consistent with, and extends, the observation that PEPCK-C mRNA expression is increased in mice with fescue toxicosis (Settivari et al., 2006). Together, the increased hepatic AST and PEPCK-C content suggests that cattle grazing HE forage had an elevated capacity for gluconeogenesis, met in part through an increased capacity to metabolize aspartate carbons for phosphoenolpyruvate production.

Because HE steers also had elevated concentrations of serum ammonia, but no difference in serum urea concentrations, the relative content of 2 enzymes (glutamine synthetase, glutamate dehydrogenase) and 3 transport-associated proteins responsible for high-affinity glutamate transport (GLT-1, EAAC1, GTRAP3–18) proteins involved with glutamine-glutamate cycle-dependent N metabolism in these tissues (Matthews, 2005) was evaluated. However, no treatment differences were observed. This finding, coupled with no treatment effect on serum urea concentrations indicates that metabolism involving proteins other than those of the hepatic urea and glutamine-glutamate cycle evaluated may be responsible for the elevation in serum ammonia concentrations found in steers grazing HE forages.

In contrast to the liver finding for AST, no treatment differences in the relative content of any evaluated protein was observed in kidney ($P > 0.15$). Because kidney tissue weight was not affected by treatment, and assuming that the proportion of cell types in kidney did not differ among treatments, these findings indicate that the kidney likely was not involved in alteration of evaluated serum metabolites.

Steers Grazing HE Forage Have Reduced BW, HCW, and Ribeye Area

To assess the cumulative effects of the altered metabolic variables on compositional gain, and to expand the limited database of carcass traits of growing steers grazing endophyte-infected forages, carcass variables of steers grazing HE vs. LE forages were compared (Table 7). After 89 to 105 d of grazing, final BW (7.4%), HCW (14%), and dressing percentage (6.9%) were less for HE than LE steers ($P \leq 0.05$). Although the ribeye area of HE steers was 15% less ($P = 0.01$) than for LE steers, ribeye area/100 kg of HCW was similar. These findings, plus the reduced dressing percentage of HE steers, indicate that smaller ribeye area of HE steers likely was representative of widespread reduction in skeletal muscle mass.

It has been observed that consumption of HE forages by preweaning calves negatively affects the ribeye area and HCW of their finished carcasses (Brown et al., 1999). In contrast, when weaned steers grazed endophyte-infected pastures during stocker through pasture-finishing production phases, dressing percentage, ribeye area, fat thickness, KPH fat, marbling, overall maturity, yield grade, and quality grade of their carcasses were not affected (Realini et al., 2005). Therefore, our carcass data begins to fill a critical void in existing

Table 6. Normalized densitometric analysis of potential treatment effects on liver, kidney, and LM content of AA enzymes and transporters in steers grazing low endophyte (LE)- or high endophyte (HE)-infected forages[1]

	Treatment				
	LE		HE		
Item[2]	Mean	SEM	Mean	SEM	*P*-value
Liver					
AST	1.00	0.29	1.56	0.48	0.008
PEPCK-C	1.00	0.37	1.90	0.39	0.001
ALT	1.00	0.52	1.28	0.64	0.319
GDH	1.00	0.32	1.08	0.35	0.609
GS	1.00	0.32	0.82	0.25	0.192
GLT-1	1.00	0.13	0.97	0.12	0.609
EAAC1	1.00	0.32	1.08	0.35	0.609
GTRAP3–18	1.00	0.46	1.50	1.26	0.273
Kidney					
AST	1.00	0.16	1.05	0.13	0.430
ALT	1.00	0.14	1.00	0.12	0.985
GDH	1.00	0.19	1.00	0.15	0.952
GS	1.00	0.22	0.86	0.18	0.147
GLT-1	1.00	0.14	1.01	0.19	0.880
EAAC1	1.00	0.12	1.00	0.20	0.978
GTRAP3–18	1.00	0.67	0.92	0.74	0.806
LM					
AST	1.00	0.27	1.11	0.29	0.417
ALT	1.00	0.24	0.89	0.28	0.371
GDH	1.00	0.44	1.19	0.36	0.333
GLT-1	1.00	0.26	1.12	0.31	0.376
EAAC1	1.00	0.15	0.93	0.16	0.311
GTRAP3–18	1.00	0.35	1.43	1.01	0.247

[1]Values (normalized arbitrary units) are arithmetic means and pooled least squares means (±SEM) of relative protein content from steers slaughtered 89 to 105 d after grazing LE (n = 9) or HE (n = 10) forages.

[2]Abbreviations: AST, aspartate aminotransferase; PEPCK-C, cystolic phosphoenolpyruvate carboxykinase; ALT, alanine aminotransferase; GDH, glutamate dehydrogenase; GS, glutamate synthetase; GLT-1, high-affinity glutamate transporter-1; EAAC1, excitatory AA carrier 1; GTRAP3–18, glutamate transport-associated protein 3–18.

data by characterizing the effect of endophyte-exposure on carcass characteristics of stocker calves, before they would be subjected to a finishing regimen.

Skeletal muscle of growing sheep and cattle is thought to be an important mediator of amino acid carbon and nitrogen balance (as reviewed by Matthews, 2005). To determine if HE-associated differences in concentrations of enzymes measured in serum also differed in skeletal muscle tissue, the LM was sampled between the 12th and 13th rib and the relative content of ALT, AST, GDH, GLT-1, EAAC1, and GTRAP3–18 evaluated by immunoblot analysis (Table 6). As with the kidney, no ($P > 0.25$) treatment differences were found in the relative content of any evaluated protein in the

Table 7. Final BW and carcass characteristics of steers grazing low endophyte (LE)- and high endophyte (HE)-infected forages[1]

	Treatment			
Item	LE	HE	SEM[2]	*P*-value
Initial BW, kg	266	267	6	0.944
Final BW, kg	338	313	12	0.051
HCW, kg	172	148	6	0.001
Dressing percentage, %	50.9	47.4	0.7	0.001
Ribeye area, cm^2	60.3	51.7	3.0	0.010
Ribeye area, $cm^2 \cdot 100\ kg^{-1}$	35.2	35.0	2.0	0.914
Backfat, cm	0.31	0.30	0.09	0.948
Backfat, $cm \cdot 100\ kg^{-1}$	0.18	0.20	0.05	0.667

[1]Data are presented as least squares means (±SEM) of steers grazing LE (n = 9) or HE (n = 10) forages for 89 to 105 d.

[2]Most conservative error of the mean.

LM of HE and LE steers. These findings suggest that the relative potential for L-Glu, L-Asp, and L-Ala metabolism mediated by ALT, AST, and GDH, nor the high-affinity uptake of L-Glu and L-Asp from blood by system X^-_{AG} proteins, were not altered by grazing of HE forages. Thus, it seems unlikely that the decreased serum ALT and AST concentrations found in HE steers were due to altered expression of these proteins by skeletal muscle tissue.

Concluding Remarks

The results of this study indicate that steers grazing tall fescue infected with high concentrations of endophyte for at least 89 d displayed classic signs of fescue toxicity. Findings were made regarding the relationship between classic serum indices of fescue toxicosis and altered expression by the liver, kidney, and skeletal muscle of several enzymes and transporters critical to interorgan nitrogen and carbon metabolism. As a consequence, this research has revealed that the potential for increased liver metabolism of L-Asp and oxaloacetate, and the shuttle of these carbons into a critical glucogenic precursor (phosphoenolpyruvate) is increased in liver, but not kidney or skeletal muscle, of steers grazing endophyte-infected tall fescue forages.

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